Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 6(4); 212-214

ISSN: 0976 822X

Research Article

Antimicrobial Activity of *Spirulina platensis* Extract Against Gram Positive and Gram Negative Bacteria- A Comparative Study

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Availble Online: 1st July, 2015

ABSTRACT
Objective: The goal of present study was focused to determine antibacterial efficacy of *Spirulina platensis* extract against few Gram positive and Gram negative bacteriae. Material and methods: Different extracts were prepared using water, methanol, ethanol and acetone. Antibacterial properties of prepared extracts were studied by well diffusion method and minimum inhibitory concentration assay. Results: Maximum zone of inhibitions were maximally shown by extract from water followed by methanol, acetone and ethanol which corresponds to 19 mm and 18 mm against *S. aureus*, *S. epidermidis*, *K. pneumonia*, *P. aeruginosa* and *E. coli* by well diffusion assay. Similarly, minimum inhibitory concentration values were depicted as 1600 $mgml^{-1}$ against *S. epidermidis* and *E. coli* against acetone extract followed by methanol extract which showed 1800 $mgml^{-1}$ in *K. pneumonia* whereas water extract showed 2025 $mgml^{-1}$ against *S. aureus*.Discussion: Our results demonstrated that incorporation of various extracts of *Spirulina platensis* have potential to inhibit the growth of both Gram positive and Gram negative bacteriae except *P. aeruginosa*, which was not shown any effective change by water extract.

Keywords: Gram positive and Gram negative bacteria, *Spirulina platensis*, Antibacterial activity, Zone of inhibition, Minimum inhibitory concentration, Water extract, Ethanol extract

INTRODUCTION

Spirulina platensis (SP) is a filamentous blue green alga which has engrossed curiosity to many researchers due to its diverse uses. It is helpful to cure many poor health conditions like diabetes, hypercholesterolemia and atherosclerosis [1-3] as well as useful in management of obesity in human subject[4]. It holds valuable compounds like polyunsaturated fatty acids (PUFA), phycocyanin and phenolics which act as antioxidants[5-7]. It is also used as nutraceutical agent due to the presence of macro and micro nutrients like carbohydrates, proteins, essential fatty acids, vitamins (B-complex, vitamin E and carotenoids), magnesium, selenium, copper, manganese, zinc and iron[8]. Several strains of blue green algae are well known for diverse biological activities such as antibacterial, antifungal, cytotoxic, algaecide, immunosuppressive[9] and antiviral activities[10]. In this regard, it had been proved that water extract of SP showed effective antibacterial activity against *S. aureus*[11]. The emergence of multiple drug resistance (MDR) has become common in world population due to disorganized uses of antibiotics. The MDR organisms compel life threatening condition through systematic infections and hence considered to be fatal to human[12-13]. Therefore, it is required as an urgent need for formulating new kind of affordable and less toxic agents. The aim of present study was to elaborate antibacterial activity of SP by using prepared extract through water, methanol, ethanol and acetone extraction process against some Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Preparation of Spirulina platensis extract

Spray dried and standard quality powder of SP was obtained from Pondicherry Spirulina Farms, Pondicherry, India for all experiments. 5 gm of SP powder was dissolved with 100 ml of distilled water in a conical flask and shaken regularly for six hours, then kept undisturbed for the next 18 hours. The macerate thus obtained was filtered with Whatman No. 1 filter paper and filtrate was collected in clean flask. About 25 ml of the filtrate was taken in a clean china bowl and allowed to dry at 40^0C. Extractive value was calculated by weighing china bowl with dry extract. The steps were repeated to prepare various extracts with different solvent like methanol, ethanol and acetone[14]. The obtained extracts were stored for further studies.

Chemicals and culture media

All chemicals used for this research work were of analytical grade and growth media that had been taken were of standard in quality. Nutrient broth, nutrient agar and Hinton Muller agar were prepared as per the instructions of manufacturer (Hi media Pvt. Ltd., India).

Bacterial cultures

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Reference bacteria required for present studies were obtained from Institute of Microbial Technology (IMTECH). The microorganisms were *S. aureus*, *S. epidermidis*, *K. pneumonia*, *P. aeruginosa* and *E. coli.*

Antibacterial activity assay

All the microorganisms were grown in sterile nutrient broth for 4 hr in incubating shaker to obtain logarithmic phase. The culture media were centrifuged to obtain a pellet then reconstituted with sterile saline. The turbidity of culture was adjusted similar to standard (adding 0.5 ml of 1% $BaCl_2$ to 99.5 ml of 0.36 N H_2SO_4). Wells were prepared using a sterile borer on Muller Hinton agar plates and 0.1 ml of broth culture was spread over the media. The plates were left undisturbed for 10 to 20 min after which four different extracts were dispensed into each well and incubated at 37°C[13-16]. The concentrations of test samples ranged were 100 $mgml^{-1}$ to 1000 $mgml^{-1}$. The presence of zone of inhibition (ZOI) surrounding the well was formed after 24 hr which has been illustrated in Table 1.

Minimum Inhibitory Concentration assay (MIC)

Various concentrations of extracts were prepared using sterile nutrient broth by serial dilutions process[13-17]. 100 µl of fresh organisms having turbidity corresponded to 1.5×10^3 CFU's were inoculated in each tube and incubated at 37°C on shaker for 24 hrs. The growth pattern was observed to determine MIC which is shown in Table 1.

Table 1: Average ZOI (mm) and MIC (mg/ml) in various extracts against Gram positive and Gram negative bacteriae.

Bacteriae	Gram reaction	Water extracts		Methanol extracts		Ethanol extracts		Acetone extracts	
		ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
S. aureus	+	19*	2025#	16	3600	13	6400	9	12800
S.epidermidis	+	19*	4050	18	3600	15	6400	18	1600#
K. pneumonia	+	18*	4050	12	1800#	15	6400	16	3200
P. aeruginosa	-	NZ	NF	19*	7200	13	6400	15	3200
E. coli	-	18	4050	6	7200	10	6400	19*	1600#

NF= not found; NZ= No zone; * = maximum inhibition; # = MIC value

RESULTS AND DISCUSSION

The results obtained from the present study using different extracts against *S. aureus*, *S. epidermidis*, *K. pneumonia*, *P. aeruginosa* and *E. coli* were recorded and analysed. It was shown that the antibacterial activity varies with extract type and its specific concentration. Since, it had been already reported that ethanol extract of SP at the dose of 1.6-1.9 $mgml^{-1}$, successfully produced clear ZOI against *E. faecalis* and *C. albicans* where as ethanol extract didn't effective against *E. Coli*, *S. typhi* and *S. aureus*[18]. Similarly methanolic extract of SP had already been observed measurable ZOI against *S. aureus* followed by *E. coli*, *P. aeruginosa and S. typhi*[19]. Extracts of water, hexane, ethyl acetate and dichloromethane had also been found effective against *S. aureus*[13, 19]. Methanol and acetone extracts of SP were efficient to observe inhibition zone against *S. aureus* and *S. typhimurium* at the dose of 250 ppm to 7000 ppm[20]. In the present study, maximum ZOI was observed with extract of water, methanol and acetone at different dose ranging from 100 $mgml^{-1}$ to 1000 $mgml^{-1}$. Ethanol extracts showed intermediate ZOI against the entire test microorganisms. MIC for water extract was 2025 $mgml^{-1}$ against *S. aureus* followed by 1600 mg/ml for acetone extract against *S. epidermidis* and *E. coli*. MIC value of 1800 mg/ml was measured for methanol extract against *K. pneumonia*. Recent studies have shown anti cancer activity[21], potential to produce biomethane and ethanol[22], ability to remove Cr (vi) from industrial waste waters[23], prevent cardiac damage caused by Tilmicosin therapy[24] and many more research which proves the multifunctional nature of SP. This report also suggests use of SP for treatment of many infectious diseases caused due to microbes.

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